DOCTORAL THESIS

Halofuginone modulates glucose metabolism and autophagy in colorectal cancer

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ABSTRACT

Cell metabolism disorder is considered as both direct and indirect consequence of oncogenic mutations for tumoigenesis. Autophagy is a metabolic stress response and a mechanism of natural cellular degradation. It is believed that autophagy, as well as metabolism, is a crucial process for the adaptation of cancer cells to changes in nutrient availability. Understanding the relationship between metabolism and autophagy and targeting on the key steps are regarded as a promising strategy to treat cancer.

Halofuginone (HF), a semisynthetic quinazolinone alkaloid originally derived from the plant *Dichroa febrifuga* Lour., has gained attention for its potential therapeutic effects in a variety of cancers. We hypothesize that HF may inhibit cancer cell proliferation by inducing changes in glucose metabolism and modulating related autophagy. A series of studies, from *in vitro* to *in vivo*, were designed to approve this concept in colorectal cancer (CRC).

Firstly, we found that HF inhibited human CRC cell proliferation and induced the generation of reactive oxygen species (ROS) and apoptosis. As expected, a reduced level of NADPH was also observed, at least in part due to inactivation of glucose-6-phosphate dehydrogenase (G6PD) in pentose phosphate pathway (PPP) upon HF treatment. Given these findings, we further investigated metabolic regulation of HF through Akt/mTORC1-mediated aerobic glycolysis and found that HF downregulated the Akt/mTORC1 signaling pathway. Moreover, metabolomics found

slower rates in both glycolytic flux and glucose-derived tricarboxylic acid cycle flux. Meanwhile, both glucose transporter GLUT1 and hexokinase-2 in glycolysis were suppressed in CRC cells by HF. These findings support our notion that HF regulates the Akt/mTORC1 signaling pathway to dampen glucose uptake and glycolysis in CRC cells. Furthermore, HF retarded tumor growth in nude mice inoculated with HCT116 cells and reduced the viability of primary cells from the tissues of CRC patients. This finding further confirmed our hypothesis that HF inhibits CRC cell growth through metabolic regulation of Akt/mTORC1.

Because mTORC1 can inhibit autophagy through phosphorylation and inactivation of the initiating kinase ULK1 in cancer cells, we further studied the HF effects on CRC in different nutritional conditions. The results showed that HF in nutrient-rich conditions could reduce SQSTM1/p62 through mTORC1-mediated phosphorylation at Ser757 of ULK1. More interestingly, HF elevated SQSTM1 protein level in low nutrient condition through AMPK-mediated phosphorylation at Ser317/777 of ULK1. It showed that HF could regulate nutrient-sensing mTORC1-ULK1 or AMPK-ULK1 to dually modulate autophagy in CRC cells. Further study by using a variety of methods, including mRFP-GFP-LC3 puncta formation, transmission electron microscope (TEM) analysis and monodansylcadaverine (MDC) staining, found that HF could induce autophagosome formation and inhibit autophagosome membrane elongation, depending on nutrient-sensing pathways. Furthermore, we found HF pronouncedly enhanced expression level of *Atg7* under nutrient-rich conditions while it decreased *Atg7* in

CRC cells under nutrient-poor conditions. These two findings imply that *Atg7* is required in dual regulation of autophagic flux depending on nutrient conditions. This conclusion was then validated by comparing with autophagy-related proteins in *Atg7* knockout (KO) MEFs and Wild-type (WT) MEFs upon HF treatment. Importantly, through analysis of metabolome and metabolic enzymes, we found that HF inhibited glycolysis under nutrient-rich conditions while it inhibited gluconeogenesis under nutrient-poor conditions in an *Atg7*-dependent manner. In subsequent studies, we found that caloric restriction (CR) in a xenograft mouse model, which mimics low nutrition *in vitro*, enhanced the anticancer activity of HF. Further analysis of the expression of TQTSM1 and LC3 in tumor tissues demonstrated that HF is an autophagic inducer in xenograft-bearing nude mice with *ad libitum* feeding, whereas it is an autophagic inhibitor when using CR.

In summary, this study explains how HF controls CRC cell growth through its influences on glucose metabolism and autophagy regulation. HF not only dually regulates autophagy *in vitro* and *in vivo* to inhibit cancer cell growth and proliferation through nutrient-sensing pathways under different conditions, but also modulates glycolysis/gluconeogenesis through an autophagic pathway. These results suggest that HF could turn out to be a potent therapeutic drug for treating CRC.

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